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DOCUMENT-IDENTIFIER: US 4709011 A
TITLE: Materials and methods for herpes simplex virus vaccination

Abstract Paragraph Left (1):
Disclosed are immunologically active preparations of Herpes simplex virus envelope glycoproteins, gD-1 and gD-2. Preferably purified through use of a monoclonal anti-gD antibody immunodsorbent, gD-1 and gD-2 preparations are incorporated in vaccines useful in generating immunological responses protective against Herpes simplex virus disease states. Disclosed also is the preparation and use in vaccination procedures of synthetic polypeptides comprising amino acid squences which are: (1) substantially common to both gD-1 and gD-2; (2) cumulatively hydrophilic in nature; (3) specifically immunoreactive with a type common, monoclonal anti-gD antibody of Group VII classification. Vaccines incorporating the synthetic polypeptides give rise to protective immunological responses, e.g., they protect mice against Herpes simplex.

Brief Summary Paragraph Right (1):
The present invention relates generally to materials and methods for developing protective responses against Herpes simplex virus ("HSV") disease states. More particularly, the present invention relates to novel preparations of HSV envelope glycoprotein gD which, when employed as the active immunogen of vaccine compositions, provoke significantly better protection in a recipient against an HSV infection disease state than heretofore obtainable in the art. The invention also relates to immunoreactive polypeptides which duplicate or substantially duplicate amino acid sequences extant in HSV gD and to the use of such polypeptides in vaccination procedures.

Brief Summary Paragraph Right (2):
Incorporated by reference herein for purposes of providing relatively current information concerning the background of the present invention is a publication of Wise, et al., "Herpes Simplex Virus Vaccines", J. Infectious Diseases, 136, pp. 706-711 (1977). Briefly summarized, this 1977 publication states that clinical illness caused by Herpes simplex virus, and especially the disability associated with recurrent infections, is a significant health problem that cannot be prevented at present. Alteration of the immune system by vaccination, it was thought, could potentially prevent or limit the infection upon subsequent exposure to the natural virus. Because such vaccination had proved efficacious in the control of many human diseases of viral etiology, an attempt to develop a vaccine against HSV was presented as a logical consideration. To accomplish this goal satisfactorily, it was noted that a number of attributes unique to the virus must be examined. These included the natural history, epidemiology, and severity of the disease, the various immune responses that were known to follow infection with the virus or immunization with experimental vaccines, and the possible risks associated with vaccine usage.

Brief Summary Paragraph Right (3):
HSV, a large, enveloped, DNA-containing virus, was noted to cause a variety of clinical entities associated with primary infection, principally involving the skin, mucosal membranes, cornea, and nervous system. The two types of HSV--type 1 (HSV-1) and type 2 (HSV-2)--were mentioned to be distinguishable by their antigenic, biologic, and biochemical characteristics. Because HSV-1 and HSV-2 differed antigenically and because an individual could have a primary infection with either type, "type-specific" HSV vaccines were stated to be a likely requirement of any vaccine development program.

Brief Summary Paragraph Right (5):
Natural infection with HSV was noted to bring into play many specific and nonspecific components of the immune defense system. Antibodies had been found to develop soon after primary infection, reach maximal levels within three to four weeks, and remain detectable for many years thereafter. Cellular immune responses to HSV infection were also detected in vivo by a delayed-type hypersensitivity response to the intradermal injection of viral antigens and in vitro by the many correlates of cellular immunity. The effects of the immune response induced by HSV upon subsequent infections in laboratory animals and humans were reported on. For example, mice immunized with either live or killed HSV, unlike unimmunized mice, were frequently found to be resistant to subsequent lethal challenge with HSV. In humans, it appeared that if individuals had preexisting HSV-1 antibodies, primary infection with HSV-2 tended to be milder. This observation and the data from studies of HSV disease in animals suggested that the immune response induced by HSV could have a beneficial effect on subsequent HSV infections and that, if a HSV vaccine could induce a similar immune response, it could ameliorate the clinical manifestations of primary HSV infections.

Brief Summary Paragraph Right (6):
Herpes simplex viruses were then noted to characteristically persist in the host and cause recurrent infections, and the disability associated with these recurrences was described as a significant health problem. The most frequent manifestations of recurrent herpetic disease states were disclosed to involve the orofacial and genital regions and recurrent herpetic keratitis was characterized as a leading cause of blindness in the United States. Herpetic genital infections with a high incidence of subsequent recurrent episodes were noted as being recognized more frequently and being associated with significant morbidity.

Brief Summary Paragraph Right (7):
The source of the virus that leads to recurrent disease was noted to be of major importance to the rationale for developing a HSV vaccine. On the basis of a variety of clinical observations, it was concluded that the virus remained dormant in nervous tissue. The isolations of HSV-1 from the trigeminal ganglia and of HSV-2 from the sacral ganglia of humans were asserted to be major steps in the further development of this concept, as were the results obtained from animal models. After extensive discussion of clinical studies of latent infections, it was generally concluded that the possibility of developing a vaccine protective against both primary infection and recurrent infection was highly remote.

Brief Summary Paragraph Right (11):
Since the time of the above-noted publication, the oncogenicity of Herpes simplex virus DNA and RNA has been the subject of confirmation by a number of investigators. See, e.g., Rapp, "Transformation by the Herpes Simplex Viruses", pp. 221-227 in "The Human Herpesviruses, An Interdisciplinary Perspective", Nahmias, et al., eds., Elsevier North Holland, Inc., New York, N.Y. (1981) and the publications cited therein. Such studies have essentially eliminated any remaining prospect for widespread use of live virus vaccines as well as those vaccine compositions including assertedly nonpathogenic, attenuated HSV strains as illustrated in U.S. Pat. No. 3,897,549.

Brief Summary Paragraph Right (12):
Consistent with the general recognition of the desirability of vaccine compositions which exclude Herpes simplex virus DNA and RNA, the number of proposals for so-called "sub-unit" vaccines has increased. See, generally, Moreschi, et al., "Prevention of Herpes Simplex Virus Infections", pp. 440-445 in "The Human Herpesvirus, An Interdisciplinary Perspective", Nahmias, et al., eds., Elsevier North Holland, Inc., New York, N.Y. (1981). As one example, U.S. Pat. No. 4,158,054 proposes, but does not exemplify, a Herpes simplex sub-unit vaccine prepared by introducing inactivated whole virus particles into continuous loading zonal ultracentrifugation provided with a density gradient containing a haemolytic surfactant followed by binding of "split" sub-units isopycnically. As other examples, there may be noted the nucleic acid freed vaccines described by: Cappel, Archives of Virology, 52, pp. 29-35 (1976); Kitces, et al., Infection and Immunity, 16, pp. 955-960 (1977); Slichtova, et al., Archives of Virology, 66, pp. 207-214 (1980); and Skinner, et al., Med. Microbiol. Immunol., 169, pp. 39-51 (1980). All the vaccine compositions of the foregoing publications were prepared by separative methodologies wherein greater or lesser care was taken to limit

or eliminate nucleic acids from the fractions extracted. None of the vaccines, however, has been found to provide uniform protection of all vaccinate test animals from death by lethal challenge with Herpes simplex virus, a generally recognized requisite for continued evaluation.

Brief Summary Paragraph Right (13):
Another Herpes simplex vaccine recently proposed and relatively thorougly tested is a composition prepared by using what is asserted to be a viral glycoprotein sub-unit fraction. In Hilleman, et al., "Sub-unit Herpes Simplex Virus-2 Vaccine" pp. 503-506 in "The Human Herpesviruses, An Interdisciplinary Perspective" Nahmias, et al., eds., Elsevier North Holland, Inc., New York, N.Y. (1981), there is proposed a mixed glycoprotein sub-unit vaccine prepared using chick embryo fibroblasts infected with type 2 Herpes simplex virus. Briefly put, the vaccine antigen is prepared through glycoprotein release by treatment of infected cells with Triton X-100, digestion with DNase, purification on a lectin affinity column, and chromatography on Sephadex. The material is then treated with formalin and formulated in alum adjuvant. Vaccinated mice are noted to be protected against lethal challenge with Herpes simplex virus type 2 to a significantly greater degree than the alum adjuvant-treated controls. The glycoprotein was less effective in reducing mortality, however, than an aqueous, UV-inactivated whole virus vaccine (which itself did not prevent death in all vaccinated animals). The ability of the glycoprotein vaccine to induce formation of both homologous and heterologous type antibodies in humans was acknowledged to be limited, and cell mediated immunity assays with respect to homologous and heterologous types indicated both limited and transitory effects.

Brief Summary Paragraph Right (14):
Of significant interest to the background of the present invention is the extensive body of information developed over the years concerning the major envelope glycoproteins of HSV. An extensive and extremely well-annotated monograph on this topic is presented in Norrild, "Immunochemistry of Herpes Simplex Virus Glycoproteins," in Current Topics in Microbiology and Immunology, 90: pp. 67-106, Springer Verlag, Berlin (1980). The major topics of discussion are: the structue, synthesis and function of HSV-specified glycoproteins; the immunological reactivity of viral membrane proteins and their components; and demonstrations of the antigenic specificities of antibodies to individual glycoproteins.

Brief Summary Paragraph Right (15):
Briefly summarized, the publication notes that Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) specify at least five major glycoproteins, designated gA, gB, gC, gD and gE, which are to be found not only in the envelope of virus particles, but in the plasma membrane of infected cells and in detergent-treated cytoplasmic extracts derived from infected cells. These glycoproteins carry strong antigen determinants that include production of antibodies in an infected host organism, and they appear to be the major immunochemical stimuli at both humoral and cellular levels in the host. Some of the viral antigen determinants are in common (i.e., gB and gD), while some are specific for one or the other of the two virus types (i.e., gC and gE). [See also, Spear, "Herpes Viruses," pp. 709-750 in "Cell Membranes and Viral Envelopes, Vol. 2," Blough, et al., eds., Academic Press, New York, N.Y. (1980)]

Brief Summary Paragraph Right (17):
The studies reported in the above-noted publications of the co-inventors and their co-workers have focused on gD of HSV-1 ("gD-1") and, in particular, on the isolation, purification and characterization of this glycoprotein. Using an extensive series of chromatographic steps, native gD-1 (previously known as CP-1 antigen) was purified in quantities sufficient to develop a monoprecipitin (or polyclonal) anti-CP-1 serum which had high titers of type-common neutralizing activity. Using anti-CP-1 as an immunological probe, it was demonstrated that gD-1 and the gD of HSV-2 ("gD-2") are both processed from lower molecular weight precursors to higher molecular weight product forms in infected cells by addition of oligosaccharides. Significant structural similarities between gD-1 and gD-2 were established by tryptic peptide analysis. Moreover, gD-1 was shown to be structurally identical whether isolated from infected human (KB) or from hamster (BHK21) cells.

Brief Summary Paragraph Right (18):
Of considerable interest were the above-noted reports of the ability of the

chromatographically purified gD-1 to provoke, in vivo, the generation of serum neutralizing antibodies which were fully protective of cells in culture against both HSV-1 and HSV-2 infections, as well as the ability of gD-1 to "block" HSV-1 and HSV-2 virus infection neutralization by protective sera.

Brief Summary Paragraph Right (19):
Finally, recent studies have described the preparation and properties of several monoclonal antibodies to HSV glycoprotein gD and other HSV glycoproteins. One report of such a study [Dix, et al., Infection and Immunity, 34: pp. 192-199 (1981)] notes that certain monoclonal antibodies to gD-1 and gC-1 were capable of use in conferring passive immunological protection against lethal challenge with HSV-1. Passive immunization with a monoclonal antibody to gD-1 (termed "HD-1") was also attributed with providing protection with a lethal challenge with HSV-2.

Brief Summary Paragraph Right (20):
Along with the above-described need for vaccine preparations for use in prevention and treatment of Herpes simplex virus disease states, there additionally exists a need for rapid and specific diagnostic tests for Herpes virus diseases and, more specifically, for antigenic substances useful in fluorescence, immunoperoxidase labelling, radioimmune and enzyme-linked immunoabsorbant assays. Such assays are commonly employed, for example, in the detection of Herpes simplex virus antibodies in samples of body fluids such as spinal fluids taken from those patients suspected of having encephalitis of Herpes simplex virus origin. See, e.g., Sever, "The Need for Rapid and Specific Tests for Herpesviruses," pp. 379-380 in "The Human Herpesviruses, An Interdisciplinary Perspective," Nahmias, et al., eds., Elsevier North Holland, Inc., New York, N.Y. (1981).

Brief Summary Paragraph Right (21):
Subsequent to the Feb. 18, 1982 filing of applicants' copending U.S. patent application Ser. No. 350,021, Watson, et al. carried out nucleic acid sequencing studies of a protein coding region of the HSV-1 (Patton strain) genome corresponding to gD-1. The results of this work appear in Science, 218, pp. 381-384 (1982). Based on the nucleic acid sequence ascertained in these studies, Watson, et al. provided a putative 394-amino acid sequence for gD-1, indicating likely glycosylation sites, designating the first twenty amino acids at the amino terminal as a putative "signal" peptide, and noting the likelihood that a series of 25 amino acids at the carboxy terminal was involved in anchoring the glycoprotein to other membrane components. DNA vectors, neither of which included the first fifty-two codons (156 bases) of the published DNA sequence, were constructed for use in microbial expression of a "gD-related" polypeptide and a .beta.-galactosidase/gD-1-related fusion polypeptide. Watson, et al. further reported that rabbits injected with the fusion protein product of E. coli expression of the fusion gene produced neutralizing antibodies to both HSV-1 and HSV-2. The directly-expressed polypeptide was not tested in vivo but was screened by immunoprecipitation assay against certain of the seventeen monoclonal antibodies screened for neutralization and RIP activity by the applicants and their co-workers in Eisenberg, et al., J. Virol., 41, pp. 478-488 (1982). The directly-expressed gD-related polypeptide was noted to be immunoprecipitable by monoclonal antibodies of Groups, I, IV and V (type common 4S, type 1 specific 1S, and RIP type 1 specific 55S and 57S) as well as polyclonal anti-HSV-1 rabbit antiserum. The polypeptide was reportedly not immunoprecipitated by monoclonals of Groups II and III (RIP type-common 12S and type-common 11S) or the group-undesignated monoclonal antibody 50S.

Brief Summary Paragraph Right (22):
The present invention provides, for the first time, an immunologically active preparation of HSV-2 envelope glycoprotein, gD-2. This glycoprotein preparation of the invention is characterized, inter alia, by its freedom from association with other HSV envelope glycoproteins, by its freedom from association with viral or cellular DNA and RNA, and by its unique immunological properties. While chromatographic procedures may be employed, the preferred procedure for isolation of gD-2 is by means of selective reversible binding to a monoclonal anti-gD antibody-containing immunoadsorbent. A preferred source of gD-2 of the invention is a cytoplasmic extract of cells infected with an HSV-2 virus. Provided also are vaccine compositions including effective amounts of gD-2 and an immunologically acceptable diluent, adjuvant or carrier, as well as vaccination procedures involving administering such vaccine compositions to

animals, including humans, for generating immunological responses protective against both HSV-1 and HSV-2 viral infection disease states. In one of its aspects, therefore, the invention provides a significant improvement in prior vaccination procedures involving administration of one or more component fractions of HSV particles for the purpose of generating a protective immunological response in a recipient animal against an HSV viral infection disease state. An antigenic mass of gD-2 is provided (in solution with an acceptable diluent, adjuvant or carrier) which is sufficient to generate an HSV-1 or HSV-2 protective response which includes formation in the host of antibodies corresponding to gD-2.

Brief Summary Paragraph Right (23):
The present invention further provides, for the first time, an immunologically active preparation of HSV-1 envelope glycoprotein, gD-1, which is distinguished from prior art preparations by isolation by selective reversible binding to a monoclonal anti-gD antibody immunoabsorbant. This glycoprotein preparation is characterized, inter alia, by immunological proper ties superior to those of the most highly purified preparations of glycoprotein gD-1 heretofore available in the art and shares with the abovenoted gD-2 preparation freedom from association with other HSV envelope glycoproteins and viral or cellular DNA. A preferred source of gD-1 of the invention is a cytoplasmic extract of cells infected with HSV-1 virus. Provided also are vaccine compositions and vaccination methods of the highly protective character and type above described with respect to gD-2 of the invention. It is similarly an aspect of the invention that significant improvements are provided in prior methods for generating protective immunological responses against HSV viral infection diseases. As with gD-2 of the invention, a novel antigenic mass of gD-1 of considerable immunological significance is provided by the invention.

Brief Summary Paragraph Right (27):
According to another aspect of the invention, immunologically active Herpes simplex virus glycoprotein D fragment replicas are provided which are suitably employed as immunoreactive materials in the manner herein described for use of the glycoproteins derived from viral sources. More specifically, the present invention provides polypeptides having amino acid sequences which duplicate in whole or part amino acid sequences extant in gD-1 and/or gD-2. Polypeptides of the invention preferably include the sequence:

Brief Summary Paragraph Right (30):
HSV-1 glycoprotein gD-1 of the invention is obtained, and HSV-2 glycoprotein gD-2 is preferably obtained, by the rapid, high yield process of purification of HSV envelope glycoprotein mixtures on a monoclonal anti-gD antibody immunoadsorbent. As previously noted, suitable sources of HSV envelope glycoproteins include the envelope of virus particles, plasma membranes of infected cells and detergent-treated cytoplasmic extracts of HSV infected cells. The last-mentioned is a preferred source. Any number of monoclonal antibody-producing hybridoma cell lines may be used as anti-gD antibody sources in developing immunoabsorbants for purification of gD-1 and gD-2 of the invention. Among the antibody producing lines which may be employed are the seventeen hybridomas described in Eisenberg, et al., J. Virol., 41, pp. 478-488 (1982). The currently preferred monoclonal cell line is HD-1 described in Dix, et al., supra. The preferred monoclonal antibody HD-1 used for purification of both gD-1 and gD-2 by immunoadsorbent-affinity chromatography had the following properties: (1) as indicated in Dix, et al., supra, it neutralized the infectivity of both HSV-1 and HSV-2 to high titers and at approximately the same levels; (2) radioimmunoprecipitation (RIP) studies showed that greater than 90% of gD remained bound to HD-1 after 2 hours of incubation at 37.degree. C.; and (3) HD-1 recognized gD in all strains of HSV-1 and HSV-2 tested. The conclusion obtained from analysis of these properties was that HD-1 recognizes a type-common antigenic determinant present on gD-1 and gD-2 and binds with a relatively high affinity. The preferred source of HD-1 antibodies is the IgG fraction of ascites fluid developed by intraperitoneal administration of HD-1 hybridoma cells to a suitable immunologically responsive animal. A preferred matrix is Sepharose 4B (Pharmacia) but other antibody immobilizing systems can be employed [See, e.g., Biotechnology Newswatch, Vol. 2, No. 2, page 3, (Jan. 18, 1982)].

Detailed Description Paragraph Right (2):
Conditions for pulse labeling of infected cells have been previously reported. For purification of gD certain modifications were made to increase the amount of label

incorporated and the amount of gD synthesized. For each experiment, ten roller bottles (490 cm.sup.2) of confluent KB or BHK cells were infected with 20 p.f.u. HSV-1 (strain HF) or 10 p.f.u. HSV-2 (SAVAGE strain). At 2 hours post infection (pi) the cells were overlaid with 50 ml of Eagle's Minimal Essential Medium (MEM) containing 5% Natal Calf serum (Dutchland Co.). At 5 hours pi the medium was decanted from one of the roller bottles and the cells were washed with warmed (37.degree. C.) Hank's salts and overlaid with 5.0 ml of Hank's salts containing the appropriate radioisotope: [.sup.35 S]-methionine (specific activity, >600 Ci/mmol) 1mCi; [2,3-.sup.3 H]arginine (specific activity 15 Ci/mmol); 1mCi. After 30 minutes the cells were overlaid with 25 ml of prewarmed complete MEM and all of the bottles were incubated for an additional 7 hours. At 12 hours pi, labeled and unlabeled cells were washed 4 times with iced saline containing 0.1 mM phenyl-methyl-sulfonyl fluoride (PMSF) and cytoplasmic extracts were prepared. To each roller bottle of cells, 5 ml of cold lysing buffer (0.01M Tris buffer, pH 7.5, containing 0.15M NaCl, 0.5% Nonidet P-40 (NP-40), 0.5% sodium deoxycholate) were added and the cells were incubated for approximately 5 minutes at 4.degree. C. Tolyl-sulfonyl phenylalanylchloromethyl ketone (TPCK) and N-.alpha.tosyl-L-lysine chloromethyl ketone (TLCK) were added, each at a concentration of 0.1 mM to inhibit proteolytic activity. The lysed cells were scraped from the bottles and centrifuged at 1200 rpm for 10 minutes to remove nuclei. The cytoplasm was centrifuged at 100,000.times.g for 1 hour. The cytoplasmic extracts were stored at -70.degree. C.

Detailed Description Paragraph Right (19):
The following example illustrates effectiveness of gD-1 vaccine composition of the invention in protection of vaccinated animals against death by massive challenge with lethal strains of both HSV-1 and HSV-2.

Detailed Description Paragraph Right (20):
The inoculant employed consisted of a solution of 1 microgram of gD-1, isolated according to Example 2 from cytoplasm of cells infected with HSV-1 strain HF, in Freund's Complete Adjuvant. Each Balb/c mouse in a first inoculated group received a total of five intraperitoneal injections over a period of two months. Seven days after the final inoculation, serum of blood taken from the retro-orbital plexis was assayed in the radioimmune precipitation (RIP) procedure of Eisenberg, et al., J. Virol., 31, pp. 608-620 (1979) and neutralizing antibody procedure of Cohen, et al., J. Virol., 10, pp. 1021-1030 (1972). All immunized animals tested positively, displaying neutralizing antibody titers of from about 1:16 to about 1:128 and immunoprecipitation results indicating production of antibodies only to gD. Because antibody immunoprecipitated both gD-1 and gD-2, it was apparent that all ten vaccinates had produced a type common neutralizing antibody to gD of HSV.

Detailed Description Paragraph Right (21):
Fourteen days after the final inoculation, the first group of 10 vaccinated mice was assembled which displayed a range of serum netralizing antibody titers (3 at .about.1:128; 3 at .about.1:64; and 4 at .about.1:32). These 10 mice, along with 9 control (unvaccinated) mice were administered intraperitoneally a dose of 4.times.10.sup.6 p.f.u. of Patton strain HSV-1 (approximately 4 times the LD.sub.50 for this strain). All control animals died within seven days, while all vaccinates were long term survivors and never appeared unhealthy.

Detailed Description Paragraph Right (24):
Four rabbits were involved in this procedure. The two vaccinated animals received slightly varying intramuscular doses of gD-1 and gD-2 prepared according to Example 2 in a vaccine composition with Freund's Complete Adjuvant. The first, gD-1, vaccinate received a total of four doses, of 10, 10 and 5 and 5 micrograms, respectively, over a period of four weeks. The second animal received doses of 9, 9, 4.5 and 4.5 micrograms of gD-2 over the same period. Each animal received a "boost" of 1 microgram of gD-1 approximately ten days later and both animals were bled three days after the boost.

Detailed Description Paragraph Right (26):
Although not substantiated by controlled experimental study, vaccines of the invention achieve effects beyond protection against disease states from post-vaccination infection of recipients in the form of limiting of ganglionic infection. Such results would be consistent with previous reports of lowered incidence of latent HSV-2 infection in animals challenged with HSV-2 after inoculation with live HSV-1. See,

e.g., McKendall, Infection and Immunity, 16, pp. 717-719 (1977). Vaccines of the invention could also be expected to limit or eliminate persistent ganglionic infection which has already been established in the recipient prior to vaccination. See, e.g., Hillemann, et al., supra, and Moreschi, et al., supra.

Detailed Description Paragraph Right (27):
While the foregoing detailed description of the invention deals with the use of Herpes simplex virus glycoproteins gD-1 and gD-2 isolated from "natural" sources, it will be understood by those skilled in the art that the present invention comprehends glycoprotein replicas, fragments of glycoproteins or fragments of glycoprotein replicas which also display the in vitro and in vivo antigenic character of the "whole" glycoprotein compounds. It is likely, for example, that effective vaccine compositions may be prepared using non-glycosylated or partially glycosylated polypeptides which themselves may be prepared by recombinant methods (see, e.g., Cohen, et al., U.S. Pat. No. 4,237,224) or even by entirely synthetic methods. [See, e.g., Zuckerman, "Developing Synthetic Vaccines", Nature, 295, No. 5845, pp. 98-99 (1982) and Dreesman, et al., "Antibody to Hepatitis B Surface Antigen After a Single Inoculation of Uncoupled Synthetic HBsAg Peptides", Nature, 295, No. 5845, pp. 158-160 (1982)].

Detailed Description Paragraph Right (30):
In the preparation of immunologically active polypeptides according to the invention, it was initially noted that the most desirable characteristics for an anti-Herpes synthetic peptide vaccine constituent would be the following: (1) it would comprise a relatively small sequence of amino acids; (2) the sequence would be a replica of a sequence common to gD-1 and gD-2; and (3) the sequence would comprise an entire, continuous antigenic determinant (epitope) rather than a portion of a conformational determinant.

Detailed Description Paragraph Right (37):
Conditions for the growth and maintenance of KB and BHK cells and the procedure used for the preparation of virus stocks of HSV-1 (strain HF) and HSV-2 (strain SAVAGE) and the plaque assay were as described previously. For infection, an input multiplicity of 20 PFU of HSV-1 or 10 PFU of HSV-2 per cell was employed.

Detailed Description Paragraph Right (38):
For the methionine, lysine and arginine radioactive labels employed, a 75 cm.sup.2 bottle of confluent KB or BHK cells was infected with HSV-1 or HSV-2. At 2 h p.i., the cells were overlaid with Eagle's minimal medium containing 1/10 the normal concentration of methionine, arginine or lysine. Pulse-labeling was carried out at 6 hours p.i. by incubating infected cells for 15 min., in 4.5 ml Hank's salts containing one of the following isotopes: [.sup.35 S]-methionine, (specific activity 600 Ci/mmole, 1 mCi); [2,3-.sup.3 H]-arginine, specific activity, 15 Ci/mmole, ImCi; [4,5-.sup.3 H]-lysine, specific activity 60-80 Ci/mmole, 1 mCi. The monolayers wee washed with iced saline, lysed, and cytoplasmic extracts prepared as described previously. For the leucine and alanine radioactive labels, infected cells were pulse-labeled at 6 hours p.i. for 15 minutes in Hank's salts, then overlaid with Eagles minimal medium and incubated at 37.degree. C. for an additional 2 hours. The following radioisotopes were used: [4,5-.sup.3 H]-leucine, specific activity 50 Ci/mmole, 1 mCi; [3-.sup.3 H]-alanine, specific activity, 75 Ci/mmole, 500 .mu.Ci.

Detailed Description Paragraph Right (43):
The general procedure used for labeling gD was to infect cells with either HSV-1 or HSV-2 then to metabolically label the cells with the particular radioactive amino acid. For methionine, arginine, and lysine a 15 min pulse carried out at 6 hrs. p.i. was sufficient to obtain enough radioactive label incorporated into gD for sequencing. Under these conditions of labeling, most of the radioactivity was found in the precursor forms of gD-1 (53,000 daltons) and gD-2 (52,000 daltons). For alanine incorporation into gD-1 and leucine incorporation into both gD-1 and gD-2, it was necessary to label for an additional 2 hours in order to get a sufficient amount of labeled gD. Under these conditions of labeling, both the precursor and product forms of the glycoproteins were labeled. At the end of the labeling period, cytoplasmic extracts were prepared and immunoprecipitated with a polyclonal antibody prepared against purified gD-1. In order to carry out sequencing studies of labeled tyrosine, gD-1 and gD-2 were purified from infected cell extracts by immunoabsorbant

chromatography and the purified proteins were iodinated with [.sup.125 I] using the Chloramine T procedure.

Detailed Description Paragraph Right (47):
Table 5 shows that the above-noted data for gD-1 can be aligned fairly well with the deduced amino acid sequence of gD-1 beginning at residue 26 of the deduced sequence. One difference is at residue 8 (33 of the deduced sequence) where the above-noted data indicates that gD-1 strain HF) contains a methionine residue. However, gD-2 (strain SAVAGE) did not. The residue predicted by nucleic acid sequencing (using strain Patton of HSV-1) is a serine. The differences noted at this position might be due to strain and type variation. However, an alteration from a methionine to a serine would require at least two base changes.

Detailed Description Paragraph Right (51):
The overall conclusion drawn from the above experiments is that gD-1 and gD-2 appear to be quite similar although not identical in sequence in the N-terminal region of the protein. Only one difference (methionine at residue 8) was noted between the Watson, et al. predicted sequence for gD-1 and the Edman degradation sequence. Since different strains of HSV-1 were used for the two studies, the data emphasize the overall conservation in sequence of gD between different strains of HSV-1.

Detailed Description Paragraph Right (56):
In the Table and throughout, the following single and triple letter "codes" for amino acid residues will be employed: A=Ala=Alanine; C=Cys=Cysteine; D=Asp=Aspartic Acid; E=Glu=Glutamic Acid; F=Phe=Phenylalanine; G=Gly=Glycine; H=His=Histidine; I=Ile=Isoleucine; K=Lys=Lysine; L=leu=Leucine; M=Met=Methionine; N=Asn=Asparagine; P=Pro=Proline; Q=Gln=Glutamine; R=Arg=Arginine; S=Ser=Serine; T=Thr=Threonine; V=Val=Valine; W=Trp=Tryptophan; and Y=Tyr=Tyrosine.

Detailed Description Paragraph Right (59):
The immunogenicity of these peptides was indirectly tested by measuring the biological reactivity of rabbit anti-peptide sera in immunoprecipitation and neutralization assays. The antisera was prepared by immunizing Female New Zealand rabbits with preparations of the synthetic peptides covalently bound to KLH (keyhole limpet hemocyanin) in 50% complete Freund adjuvant (CFA) at five weekly intervals by intramuscular injection. A total of 350 .mu.g of each of the peptides was used.

Detailed Description Paragraph Right (62):
The HSV infectivity neutralization activity of rabbit antipeptide sera was determined for gD-1, gD-2 and each of the seven synthetic peptides described above. For each of these peptides a HSV-1 and HSV-2 neutralization titer was calculated. The neutralization titer is the reciprocal of the highest dilution of antiserum which gave a 50% reduction in plaques. All of the rabbit sera were assayed using HSV-1 (strain HF) and HSV-2 (strain SAVAGE).

Detailed Description Paragraph Right (63):
For each rabbit, a pre-immunization bleeding was tested and in all cases, the neutralization titer for both HSV-1 and HSV-2 was <20. Two rabbits were immunized with each of the glycoproteins peptides and the neutralization titers of several bleedings were determined. Table 8 below provides average neutralization titer for the anti-peptide sera. Average values shown do not in any instance vary more than one dilution from any individual titers. Data are shown in Table 8.

Detailed Description Paragraph Right (73):
While the foregoing description of the invention has focused on the utility of immunologically active gD-1 and gD-2 preparations and immunologically active polypeptides as components of vaccine compositions, it will be understood that these preparations will additionally possess utility as components of highly specific diagnostic reagents for detection of Herpes simplex virus antibodies in body fluids including spinal fluids. The specific antigens of the invention (and their biologically active fragments and replicas) may be used to sensitize inert particles of types well known in the art as useful in diagnostic, antigen-antibody reaction detection schemes. In this regard, antigen preparations and antigen-sensitized particles of the invention may be used in combination with suitable "marker" substances (either chemical or radiochemical) in the detection of antibodies by

agglutination and radioimmunoassay, as well as fluorescence and enzyme immunoassay, techniques.

Detailed Description Paragraph Table (3):

TABLE 3 Prepared Antiserum Against Mouse Neutralization Titer.sup.a Designation Number HSV-1 HSV-2
anti-gD-1 1 2048 1536 2 1536 512 3 1536 512 anti-gD-2 1 192 512 2 512 1024 3 1024 1024 4 1024 1536 5 192 512
.sup.a Results are expressed as the reciprocal of the greatest dilution o serum resulting in a 50% reduction of p.f.u. as compared with appropriate virus and preimmune mouse serum controls (22). AntiCP-1 serum (rabbit) ha a neutralization titer of 512 against HSV1 and 256 against HSV2 when tested in the same assay system.

Detailed Description Paragraph Table (4):

TABLE 4 Concentration of gD Required.sup.a Virus Employed gD-1 gD-2
HSV-1 37 ND.sup.b HSV-2 35 30
.sup.a ng of gD required to block by 50% the capacity of antiCP-1 serum t neutralize 30 p.f.u. of either HSV1 or HSV2 .sup.b Not determined

Detailed Description Paragraph Table (6):

TABLE 6

```
gD-1 KYALADASLKMADPNRFRGKDLPVLDQLTDPPGVRRVYHI 40 gD-2
------P----------N----------K----- 80 gD-1
QAGLPDPFQPPSLPITVYYAVLERACRSVLLNAPSEAPQI 80 gD-2
-PS-E-------I-----------------H-------- gD-1 VRGASEDVRKQPYNLTIAWFRMGGNCAIPITVMEYTECSY
120 gD-2 -----DEA--HT---- ---Y---D----------P- gD-1
NKSLGACPIRTQPRWNYYDSFSAVSEDNLGFLMHAPAFET 160 gD-2
-----V---------S---------------------- gD-1 AGTYLRLVKINDWTEITQFILEHRAKGSCKYALPLRIPPS
200 gD-2 ----------------------RA-------- ----A gD-1
ACLSPQAYQQGVTVDSIGMLPRFIPENQRTVAVYSLKIAG 240 gD-2
---TSK-------------------------L------- gD-1 WHGPKAPYTSTLLPPELSETPNATQPELAPEDPEDSALLE
280 gD-2 -----P----------D-T--------V---------- gD-1
DPVGTVAPQIPPNWHIPSIQDAATPYHPPATPNNMGLIAG 320 gD-2 --A---SS------
-------V---H-A--A-S-P---I- 319 gD-1 AVGGSLLAALVICGIVYWMHRRTRKAPKRIRLPHIREDDQ 360 gD-2
-LA--T------G--AF-VR--AQM----L------D--A 359 gD-1 PSSHQPLFY 369 gD-2 -P------- 368
```

Detailed Description Paragraph Table (8):

TABLE 8 Rabbit Serum Neutralization of HSV Infectivity Rabbit Antiserum HSV-1 HSV-2
gD-1 1600 800 gD-2 1200 1600 8-23[1] 32 16 8-23[2] <20 <20 1-16[1] <20 <20 1-16[2] 20 30 1-23[H] 80 40 1-23[1] 40 80 1-23[2] 40 120

Detailed Description Paragraph Table (9):

TABLE 9 Protection of Mice Against a Lethal IP Challenge by HSV-2 After Immunization With gD-1 or Synthetic Peptides Neutralization Titer Number Number Exp. No. Immunogen HSV-1 HSV-2 Challenged Dead
1 KLH 6 6 20 16 gD-1 800 400 10 0 8-23[1] 40 10 10 2 1-23[H] 30 10 10 2 2 KLH <6 <6 19 13 gD-1 384 192 10 1 1-23[H] 384 192 10 2 3 KLH 6 <6 9 9 8-23[2] 6 <6 10 7 8-23[1] 384 256 10 2 1-23[H] 388 239 10 7 1-23[2] 15 69 10 1 4 KLH 6 <6 10 10 gD-1 548 338 10 0 1-23[H] 388 69 10 6 1-23[2] 11 26 10 4 1-23[1] 478 223 10 2

Detailed Description Paragraph Table (10):

TABLE 10 Protection of Mice Against a Paralytic Challenge by HSV-2 Given by the Foodpad Route After Immunization With gD-1 or Synthetic Peptides Number Para- Neutralization Number lyzed Titer Chal- or Exp. No. Immunogen HSV-1 HSV-2 lenged Dead
1 KLH <6 <6 10 10 gD-1 512 181 10 0 1-23[H] 388 104 10 0 CFA <6 <6 10 3 2 KLH 6 <6 10 9 gD-1 534 446 10 0 1-23[H] 169 128 10 2 1-23[2] 14 73 10 2 1-23[1] 512 382 10 0 3 CFA ND* ND 10 10 1-23[1] CFA 256 111 5 0 AP ND ND 10 10 1-23[1] AP 338 158 5 0

*ND = Not determined.

CLAIMS:

1. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Try-Ala-Leu-Ala-Asp-Ala-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-COOH.

2. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-Gly-Lys-Asp-Leu-Pro-COOH.

3. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Tyr-Ala-Leu-Ala-Asp-Ala-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-G
ly-Lys-Asp-Leu-Pro-COOH.

4. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Tyr-Ala-Leu-Ala-Asp-Pro-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-COOH.

5. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-Gly-Lys-Asn-Leu-Pro-COOH.

6. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Tyr-Ala-Leu-Ala-Asp-Pro-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-G
ly-Lys-Asn-Leu-Pro-COOH.

7. A polypeptide suitable or use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Tyr-Ala-Leu-Ala-Asp-Pro-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-G
ly-Lys-Asp-Leu-Pro-COOH.

8. A polypeptide suitable for use in a vaccination procedure for generating an immunological response protective against a Herpes simplex virus disease state, said polypeptide comprising the following amino acid sequence:

NH.sub.2 -Lys-Tyr-Ala-Leu-Ala-Asp-Ala-Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-Arg-Phe-Arg-G
ly-Lys-Asp-Leu-Pro-Val-Leu-Asp-Gln-Leu-Thr-Asp-COOH.

1: J Immunol 1994 Aug 15;153(4):1858-68 Related Articles, NEW Books, LinkOut

Targeted lymph node immunization with simian immunodeficiency virus p27 antigen to elicit genital, rectal, and urinary immune responses in nonhuman primates.

Lehner T, Bergmeier LA, Tao L, Panagiotidi C, Klavinskis LS, Hussain L, Ward RG, Meyers N, Adams SE, Gearing AJ, et al.

Department of Immunology, United Medical School, Guy's Hospital, London, United Kingdom.

A s.c. route of immunization was developed in non-human primates, which targets the genitourinary-rectal associated lymphoid tissue. A vaccine consisting of rSIV gag p27, expressed as hybrid Ty virus-like particles (p27: Ty-VLP) was administered in the proximity of the internal iliac lymph nodes. Secretory IgA and IgG Abs to the p27 Ag were elicited in the vaginal, male urethral, rectal and seminal fluids, urine and serum. Two or more immunodominant B cell epitopes were identified within peptides 51-90 and 121-170 of the sequence of p27, using serum or biliary IgA and IgG Abs. CD4+ T cell proliferative responses to p27 were elicited predominantly in the targeted internal iliac, as well as the inferior mesenteric lymph nodes and the spleen, but not in the unrelated lymph nodes. These cells were then studied for helper function in p27 specific B cell Ab synthesis. Specific IgA and IgG Abs were detected in the same lymphoid tissues as those that displayed proliferative responses. However, cross-over reconstitution experiments between splenic and iliac lymph node B and CD4+ T cells suggest that the iliac B cells are essential for specific IgA Ab synthesis, whereas splenic B cells preferentially synthesize IgG Ab. The targeted lymph node (TLN) route of immunization gave comparable B cell, proliferative T cell, and Th cell responses to the vaginal, male genitourinary, and rectal mucosal routes, which were augmented by oral immunization. However, the TLN route induced urinary and seminal fluid sIgA and IgG Abs in addition to genital and rectal Abs. Generating secretory IgA and IgG Abs at the mucosal surfaces, and T and B cell immunity in the regional draining lymph nodes, spleen and circulation by TLN immunization may prevent transmission of virus through the mucosa, dissemination of the virus, and the formation of a latent reservoir of infection.

PMID: 7519218 [PubMed - indexed for MEDLINE]

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L9: Entry 5 of 56 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277621 B1
TITLE: Artificial chromosome constructs containing foreign nucleic acid sequences

Brief Summary Paragraph Right (3):
Herpes Simplex virus (HSV) is the prototypic human herpes virus. Despite the fact that HSV is a human pathogen, there has been a great deal of interest in using HSV as a therapeutic agent. The HSV genome has been sequenced, and many HSV mutants have been generated and used specifically in this context. Generation of HSV mutants has been carried out by using drug selection or by co-transfection of cells with plasmid DNA, usually modified by insertion of a marker gene, and intact viral DNA. Mutants are identified by screening for either drug resistance or recombination and expression of the marker gene, or by plaque hybridization. Another method that has been used to generate herpes virus mutants involves the use of cosmid sets that, taken together, contain complete herpes virus genomes. For example, cosmid sets that contain the entire genomes of Pseudorabies virus (PRV), Varicella-Zoster virus (VZV), Herpes Simplex virus (HSV), Cytomegalovirus (CMV), and Epstein-Barr virus (EBV) have been created. In constructing complete viral genomes from these cosmids, viral sequences are released from the cosmid backbones and transfected into cells. Viral plaques are produced via recombination between the overlapping fragments, which together represent the entire genome. Specific mutations are made in the viral genomes by manipulating the cosmid DNA.

Brief Summary Paragraph Right (5):
Accordingly, in one aspect, the invention features an artificial chromosome construct containing a nucleic acid sequence that directs formation of a recombinant virus (e.g., a lytic or a non-lytic virus) upon introduction into a cell. Optionally, the artificial chromosome construct, either in the artificial chromosome portion or in the nucleic acid sequence portion, further includes a heterologous nucleic acid sequence that, for example, encodes a therapeutic gene product, such as a growth factor, a hormone, an enzyme, a vaccine antigen, a cytotoxin, an immunomodulatory protein, an antisense RNA molecule, or a ribozyme. The artificial chromosome portion of the construct can be derived from a bacterial artificial chromosome, a P1-derived artificial chromosome, a yeast artificial chromosome, or a mammalian (e.g., human) artificial chromosome. The recombinant virus encoded by the nucleic acid sequence included in the artificial chromosome construct can be a herpes virus, such as a herpes simplex virus. Other viruses that can be encoded by the nucleic acid sequence are listed below.

Drawing Description Paragraph Right (1):
FIG. 1 is a schematic representation of the HindIII fragment of plasmid BAC-TK. Fragments AB and CD correspond to nucleotides 47860-47150 and 47057-45582, respectively, of the HSV-1 strain 17 genome (Genbank accession numbers X14112, O00317, O00374, and S40593). "H" represents HindIII restriction sites, and "cm" and "tk" represent chloramphenicol and thymidine kinase coding sequences, respectively.

Detailed Description Paragraph Right (3):
Viral nucleic acid sequences that can be inserted into artificial chromosomes to generate the artificial chromosome constructs of the invention can be derived from any of a number of well known viruses, such as viruses that include a circular replication intermediate. For example, members of DNA virus families, e.g., the Herpeseviridae (e.g., HSV-1, HSV-2, VZV, CMV, EBV, HHV6, or HHV7), Adenoviridae, Poxviridae, Papovaviridae (e.g., papillomaviruses and polyomaviruses), Parvoviridae, and Hepadnaviridae families, can be used. Members of RNA virus families, the genomes of

which can be made into DNA by standard molecular techniques, can also be used. For example, members of the Coronaviridae, Picornaviridae, Retroviridae, Caliciviridae, Togaviridae (e.g., flaviviruses), and Astroviridae families, which are single, plus stranded viruses, can be used. Also, members of the Paramyxoviridae, Orthomyxoviridae, Filoviridae, Rhabdoviridae, Arenaviridae, and Bunyaviridae families, which are single, negative stranded viruses, can be used. Double stranded RNA viruses, such as those of the family Reoviridae, can also be used in the invention.

Detailed Description Paragraph Right (14):
Examples of non-tissue specific promoters that can be used in the invention include the early Cytomegaloviris (CMV) promoter (U.S. Pat. No. 4,168,062) and the Rous Sarcoma Virus promoter (Norton et al., Molec. Cell Biol. 5:281, 1985). Also, HSV promoters, such as HSV-1 IE and IE 4/5 promoters, can be used.

Detailed Description Paragraph Right (15):
Examples of tissue-specific promoters that can be used in the invention include, for example, the desmin promoter, which is specific for muscle cells (Li et al., Gene 78:243, 1989; Li et al., J. Biol. Chem. 266:6562, 1991; Li et al., J. Biol. Chem. 268:10403, 1993); the enolase promoter, which is specific for neurons (Forss-Petter et al., J. Neuroscience Res. 16(1):141-156, 1986); the .beta.-globin promoter, which is specific for erythroid cells (Townes et al., EMBO J. 4:1715,1985); the tau-globin promoter, which is also specific for erythroid cells (Brinster et al., Nature 283:499, 1980); the growth hormone promoter, which is specific for pituitary cells (Behringer et al., Genes Dev. 2:453, 1988); the insulin promoter, which is specific for pancreatic beta cells (Selden et al., Nature 321:545, 1986); the glial fibrillary acidic protein promoter, which is specific for astrocytes (Brenner et al., J. Neurosci. 14:1030, 1994); the tyrosine hydroxylase promoter, which is specific for catecholaminergic neurons (Kim et al., J. Biol. Chem. 268:15689, 1993); the amyloid precursor protein promoter, which is specific for neurons (Salbaum et al., EMBO J. 7:2807, 1988); the dopamine .beta.-hydroxylase promoter, which is specific for noradrenergic and adrenergic neurons (Hoyle et al., J. Neurosci. 14:2455, 1994); the tryptophan hydroxylase promoter, which is specific for serotonin/pineal gland cells (Boularand et al., J. Biol. Chem. 270:3757, 1995); the choline acetyltransferasc promoter, which is specific for cholinergic neurons (Hersh et al., J. Neurochem. 61:306, 1993); the aromatic L-amino acid decarboxylase (AADC) promoter, which is specific for catccholaminergic/5-HT/D-type cells (Thai et al., Mol. Brain Res. 17:227, 1993); the proenkephalin promoter, which is specific for neuronal/spermatogenic epididymal cells (Borsook et al., Mol. Endocrinol. 6:1502, 1992); the reg (pancreatic stone protein) promoter, which is specific for colon and rectal tumors, and pancreas and kidney cells (Watanabe et al., J. Biol. Chem. 265:7432, 1990); and the parathyroid hormone-related peptide (PTHrP) promoter, which is specific for liver and cecum tumors, and neurilemoma, kidney, pancreas, and adrenal cells (Campos et al., Mol. Rnfovtinol. 6:1642, 1992).

Detailed Description Paragraph Right (18):
Alternatively, the construct can be administered via a parenteral route, e.g., by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route, or via a mucosal surface, e.g., an ocular, intranasal, pulmonary, oral, intestinal, rectal, vaginal, or urinary tract surface. An artificial chromosome construct formulated in association with bupivacaine (see below) is advantageously administered into muscle tissue. When a neutral or anionic liposome or a cationic lipid, such as DOTMA or DC-Chol (see below), is used, the formulation can be advantageously injected via intravenous, intranasal (aerosolization), intramuscular, intradermal, or subcutaneous routes. An artificial chromosome construct in a naked form can advantageously be administered via intramuscular, intradermal, or subcutaneous routes.

Detailed Description Paragraph Right (29):
We used bacterial artificial chromosome (BAC) technology to clone the entire HSV genome. A plasmid, BAC-TK, was constructed with viral tk sequences flanking the signals necessary for chromosomal maintenance in bacteria and the chloramphenicol resistance gene (FIG. 1). This plasmid was linearized and co-transfected with HSV-1 infectious DNA into Vero cells (ATCC CRL 1587). The resultant virus stocks were screened with 100 .mu.M acyclovir (ACV), and resistant viruses were isolated and plaque purified. Southern blot and PCR analysis confirmed insertion of BAC and

chloramphenicol sequences within the tk locus. The recombinant virus was designated HSV-BAC-TK.

Detailed Description Paragraph Right (35):
Vectors based on HSV have been used extensively for gene transfer. HSV-plasmid vectors (amplicons) contain a eukaryotic expression cassette and the HSV signals for replication and packaging. The amplicon vector is replicated and packaged into virions when co-propagated with a helper virus, either wild type virus, replication-defective virus, or virus generated from HSV cosmids. In fact, a modified cosmid set has been used to produce amplicon preparations that are free of contaminating helper virus (Fraefel et al., supra). However, herpes virus cosmids are prone to deletion and rearrangement and, further, are often heterogeneous in nature. A BAC clone of HSV overcomes these problems.

Detailed Description Paragraph Right (39):
Plasmid pTK-AB was created by subcloning the Bg1II/EcoRI fragment from pXhoIf (the XhoI F fragment of HSV-1 strain 17 cloned into plasmid pAT153) into the BamHI/EcoRI sites of pHSV1ac (Geller et al., stpra). The tk-containing plasmid, BH13 (Horsburgh et al., Cell 86(6):949-959, 1996) was digested with PstI and XhoI, to release the 3' end of the tk gene, and the fragment was subcloned into the PstI/XhoI site of pSC 1180, creating plasmid TK-CD. Plasmids TK-AB and TK-CD were digested with HindIII/MscI and HindIII/Sa1I, respectively, and the resulting fragments were subcloned into the HpaI/Sa1I sites of pBeloBAC (Dr. H. Shizuya and Dr. M. Simon, Department of Biology, California Institute of Technology, Pasadena, Calof.) to create plasmid pBAC-TK (FIG. 1).

Detailed Description Paragraph Right (42):
Plasmid BAC-TK was digested with HindIII and co-transfected into Vero cells with infectious HSV-1 F strain DNA, as described by Chiou et al. (Virology 145(2):213-226, 1985). DNA from plaques that were resistant to 100 .mu.M acyclovir (ACV) was screened by PCR using primers that correspond to chloramphenicol sequences (Table 1). From each of two independent transfections, one recombinant virus was plaque-purified two times and the mutation confirmed by Southern blot hybridization. This virus was designated HSV-BAC.

Other Reference Publication (2):
BC Horsburgh et al., Allele replacement: an application that permits rapid manipulation of herpes simplex virus type 1 genomes, Gene Therapy, 6, 922-930 (1999).

Other Reference Publication (4):
Y. Saeki et al., Herpes Simplex Virus Type 1 DNA Amplified as Bacterial Artificial Chromosome in Eschericia coli; Rescue of Replication-Compentent Virus Progeny and Packaging of Amplicon Vectors, Human Gene Therapy, 9:2787-2794 (Dec. 10, 1998).

Other Reference Publication (8):
Chiou et al., "Mutations in the Herpes Simplex Virus Major DNA-Binding Protein Gene Leading to Altered Sensitivity to DNA Polymerase Inhibitors", Virology, 145:213-226 (1985).

CLAIMS:

5. The artificial chromosome construct of claim 1, wherein said recombinant virus is a herpes virus.

8. The artificial chromosome construct of claim 5, wherein said herpes virus is a herpes simplex virus.

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L9: Entry 10 of 56 File: USPT Apr 3, 2001

US-PAT-NO: 6210663
DOCUMENT-IDENTIFIER: US 6210663 B1

TITLE: Methods of augmenting mucosal immunity through systemic priming and mucosal boosting

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ertl; Hildegund C. J.	Villanova	PA		

US-CL-CURRENT: 424/93.2; 424/184.1, 424/208.1, 424/229.1, 424/249.1, 435/320.1

CLAIMS:

What is claimed is:

1. A method of enhancing mammalian mucosal immunity to the repeated presentation of a viral antigen from a virus which can infect said mammal via the mucosal tissue, said method comprising the steps of:

(a) administering via an intramuscular or intradermal route of administration an effective amount of a priming DNA composition which comprises a plasmid comprising a DNA sequence encoding said viral antigen under the control of regulatory sequences directing expression thereof in a mammalian cell; and

(b) subsequently administering intranasally to said mammal an effective amount of a boosting composition which comprises a recombinant adenovirus comprising a nucleotide sequence encoding said antigen under the control of a regulatory sequence directing expression thereof in a mammalian cell,

wherein the immune responses induced to said antigen are enhanced in serum and at a mucosal site, and wherein mammalian immune responses to the adenovirus antigens of said recombinant adenovirus are reduced.

2. The method according to claim 1, wherein said priming DNA composition comprises a suitable physiologically acceptable carrier.

3. The method according to claim 1, wherein said effective amount of said priming composition ranges between about 1 .mu.g to about 10,000 .mu.g of said plasmid.

4. The method according to claim 1, wherein said priming amount is administered intramuscularly 2 to 27 weeks before administering said boosting composition.

5. The method according to claim 1, wherein said recombinant adenovirus is replication defective.

6. The method according to claim 1, wherein said mammal has pre-existing immunity to adenovirus.

7. The method according to claim 5, wherein said boosting amount ranges from about 10.sup.4 to about 10.sup.16 pfu of said recombinant adenovirus.

8. The method according to claim 1, wherein said virus is selected from the group consisting of a virus that causes a sexually transmitted disease, a virus that causes an upper respiratory infection, and a virus that causes an intestinal infection.

9. The method according to claim 8, wherein said virus is selected from the group consisting of herpes simplex virus types 1 and 2, human immunodeficiency virus, and human papilloma virus, and respiratory syncytial virus.

10. The method according to claim 1, wherein said method further comprises co-administeing with said priming composition a genetic adjuvant selected from a group consisting of interleukin-4, interleukin-5, and interleukin-12 expressed by a plasmid-based vector.

11. The method according to claim 10, wherein said genetic adjuvant is interleukin-5.

12. A kit for inducing mucosal immunity in mammals, said kit comprising (a) a priming composition in an intramuscular or intradermal formulation which comprises a plasmid comprising a DNA sequence encoding a viral antigen from a virus which can infect said mammal via the mucosal tissue and which is under the control of regulatory sequences directing expression thereof in a mammalian cell; (b) a boosting composition in an intranasal formulation which comprises a recombinant adenovirus comprising said DNA sequence encoding said antigen under the control of a regulatory sequence directing expression thereof in a mammalian cell; and (c) instructions for the use of said kit.

13. The kit according to claim 12, wherein said kit further comprises a composition encoding a genetic adjuvant selected from a group consisting of interleukin-4, interleukin-5, and interleukin-12.

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L9: Entry 5 of 56 File: USPT Aug 21, 2001

US-PAT-NO: 6277621
DOCUMENT-IDENTIFIER: US 6277621 B1

TITLE: Artificial chromosome constructs containing foreign nucleic acid sequences

DATE-ISSUED: August 21, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Horsburgh; Brian	Vancouver			CAX
Qiang; Dong	Vancouver			CAX
Tufaro; Francis	Vancouver			CAX
Ostrove; Jeffrey	West Vancouver			CAX

US-CL-CURRENT: 435/235.1; 435/320.1, 435/325, 435/366, 536/23.1, 536/23.2, 536/23.5, 536/23.51, 536/23.52, 536/23.72, 536/24.5

CLAIMS:

What is claimed is:

1. A bacterial artificial chromosome construct comprising a nucleic acid sequence that directs formation of a recombinant virus upon introduction into a cell.

2. The artificial chromosome construct of claim 1, wherein said recombinant virus is a lytic virus.

3. The artificial chromosome construct of claim 1, wherein said recombinant virus is a non-lytic virus.

4. The artificial chromosome construct of claim 1, wherein said artificial chromosome or said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

5. The artificial chromosome construct of claim 1, wherein said recombinant virus is a herpes virus.

6. The artificial chromosome construct of claim 4, wherein said heterologous nucleic acid sequence encodes a therapeutic gene product.

7. The artificial chromosome construct of claim 6, wherein said therapeutic gene product is selected from the group consisting of growth factors, hormones, enzymes, vaccine antigens, cytotoxins, immunomodulatory proteins, antisense RNA molecules, and ribozymes.

8. The artificial chromosome construct of claim 5, wherein said herpes virus is a herpes simplex virus.

9. A cell comprising a bacterial artificial chromosome construct stably integrated into its genome.

10. The cell of claim 9, wherein said artificial chromosome construct comprises a nucleic acid sequence that encodes an HSV genome in which an immediate early gene comprises a mutation.

The effect of route of immunization on mucosal immunity and protection.

Lehner T, Wang Y, Ping L, Bergmeier L, Mitchell E, Cranage M, Hall G, Dennis M, Cook N, Doyle C, Jones I.

Department of Immunobiology, United Medical and Dental Schools at Guy's Hospital, London, SE1 9RT, United Kingdom. t.lehner@umds.ac.uk

In macaques, the route of immunization has a profound effect on the immune response. Augmenting rectal or vaginal immunization with oral or nasal immunization enhanced the secretory IgA, serum IgG, and T cell responses. However, targeted iliac lymph node (TILN) immunization with recombinant simian immunodeficiency virus (SIV) gp120 and p27 elicited the most consistent mucosal antibody responses in the rectum, vagina, urine, seminal fluid, and blood. Both mucosal and TILN immunization induced specific CD4+ T cell proliferative responses in the iliac lymph nodes, which drain these mucosal surfaces, and in the splenic and circulating T cells. Rectal mucosal challenge with cell-free SIV induced total protection in 4 of 7 macaques that were immunized by the TILN route, and, compared with unimmunized macaques or those immunized by the mucosal route ($P<.001$), it induced a >90% decrease in virus load in 3 of them. Protection from mucosal rectal infection with SIV was significantly associated with an increase in the CD8 suppressor factor (which was generated by the iliac lymph node), RANTES, and MIP-1beta ($P<.01$).

Publication Types:
- Review
- Review, Tutorial

PMID: 10099126 [PubMed - indexed for MEDLINE]

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L9: Entry 47 of 56 File: USPT Aug 5, 1997

DOCUMENT-IDENTIFIER: US 5654174 A
TITLE: Herpes simplex virus glycoprotein D variants

Abstract Paragraph Left (1):
The present invention provides variant HSV-1 glycoprotein D and HSV-2 glycoprotein D molecules capable of preventing infection of cells by herpes simplex virus types 1 and/or 2. Also provided are novel purified and isolated polynucleotides encoding the variant gD molecules. HSV gD-1 and gD-2 region IV variants or fragments thereof are specifically contemplated by the invention. The presently preferred variant molecule gD-1(.DELTA.290-299t) is the product of recombinant expression in Sf9 cells of a fusion protein including the signal peptide of honeybee melittin and Patton strain HSV-1 gD wherein (1) the Patton strain amino acid residues 290 through 299 of the mature gD-1 protein have been replaced with the amino acid residues arginine, lysine, isoleucine and phenylalanine, and (2) Patton strain amino acid residues 308 through 369 have been replaced with five histidine residues. When expressed in Sf9 cells, cleavage of the melittin signal peptide results in the presence of aspartate and proline residues at the amino terminus of the variant molecule. The amino acid sequence of gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 2 and the preferred DNA sequence encoding gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 1. Administration of gD variant molecules of the invention to mammalian subjects, especially humans, for the purpose of preventing HSV infection and/or ameliorating pathological sequelae of HSV infection is specifically contemplated.

Brief Summary Paragraph Right (1):
The present invention relates generally to novel herpes simplex virus glycoprotein D molecules. More particularly, the present invention relates to variant glycoprotein D molecules which are capable of blocking infection of cells by herpes simplex virus.

Brief Summary Paragraph Right (2):
Herpes simplex viruses (HSV) are human pathogens which cause a variety of disease states including cold sores, eye and genital infections, life-threatening neonatal infections, and encephalitis. HSV is also capable of establishing latent infections in ganglia. The strains designated HSV-1 (oral) and HSV-2 (genital) are members of the family Herpesviridae and are classified in the subfamily alphahexpesvirinae and the genus simplex virus. The viruses have an enveloped double-stranded DNA genome of 150 kilobases (kb) including at least seventy-two open reading frames which encode at least eleven glycoproteins. The genomes of HSV-1 and HSV-2 exhibit extensive homology in regions which are known to encode proteins responsible for antigenic specificity and/or biological activity.

Brief Summary Paragraph Right (3):
Upon infection, several vital glycoproteins act singly or in concert to bind HSV to a susceptible cell and trigger direct fusion between the virion envelope and the cell membrane. Glycoprotein D (gD) of HSV is a component of the virion envelope which plays an essential role in HSV entry into susceptible mammalian cells. The evidence to date suggests that gD binds to a cellular molecule, possibly the mannose-6-phosphate receptor, following the initial interaction of HSV glycoproteins gC and gB with heparan sulfate proteoglycans. The interaction between gD and its receptor may stabilize the virus-cell complex prior to membrane fusion which is mediated by other essential glycoproteins such as gB, gH, and gL. See Sisk et al., J. Virol., 68(3): 766-775 (1994) and Tat-Singer et al., J. Virol., 69(7): 4471-4483 (1995). The nucleotide sequence of the Patton strain of HSV-1 gD (gD-1) (SEQ ID NO: 3) was first reported in Watson et al., Science, 218:381-384 (1982). The strain 333 HSV-2 gD (gD-2)

was described in Muggeridge et al., J. Virol., 64(8): 3617-3626 (1990). The nucleotide sequence of the strain 333 gD-2 gene is set out in SEQ ID NO: 14 herein.

Brief Summary Paragraph Right (7):
The present invention provides variant HSV gD molecules capable of preventing infection of cells by HSV-1 and/or HSV-2. Also provided are novel purified and isolated polynucleotides (i.e., DNA and RNA both sense and antisense strands) encoding the variant gD molecules. HSV gD-1 and gD-2 region IV variants or fragments thereof are specifically contemplated by the invention.

Brief Summary Paragraph Right (9):
The presently preferred gD-1 variant molecule, designated gD-1 (.DELTA.290-299t), is the product of recombinant expression in Sf9 cells of a fusion protein including the signal peptide of honeybee melittin [Tessier et al., Gene, 98:177-183 (1991)] and Patton strain HSV-1 gD wherein (1) the Patton strain amino acid residues 290 through 299 of the mature gD-1 protein have been replaced with the amino acid residues arginine, lysine, isoleucine and phenylalanine, and (2) Patton strain amino acid residues 308 through 369 have been replaced with five histidine residues. When expressed in Sf9 cells, cleavage of the melittin signal peptide results in the presence of aspartate and proline residues at the amino terminus of the variant molecule. The amino acid sequence of gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 2 and the preferred DNA sequence encoding gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 1.

Brief Summary Paragraph Right (15):
Administration of gD variant molecules or fragments thereof to mammalian subjects, especially humans, for the purpose of preventing 1HSV infection and/or ameliorating pathological sequelae of HSV infection is specifically contemplated. Various animal models for HSV infection are accepted in the art and include, but are not limited to, the rabbit and mouse eye models of herpes keratitis [Hill et al., Curr. Eye Res., 6:1065-1071 (1987) and Rock and Fraser, Nature, 302: 523-525 (1983)], cutaneous herpes infection of hairless (nude) mice [Metcalf et al., Infect. Immunol., 26:1164-1171 (1979)], vaginal lesions in the guinea pig and mouse [Stanberry et al., J. Infect. Dis., 146:397-404 (1982)], foot pad model in mice [Stevens and Cook, Science, 173:843-845 (1971)], zosterform skin model in mice [Hill et al., J. Gen. Virol., 39:21-28 (1982)], and experimental herpes simplex encephalitis induced by intracerebral vital inoculation in mice [Tenser, J. Infect. Dis., 147:956 (1983)]. For review, see Stevens, Microbiol. Rev., 53:318-332 (1989) and Stanberry, Current Topics in Microbiol. and Immunol., 179:15-30 (1992). The gD variant molecules are administered to the mammal in an amount sufficient to block infection of susceptible cells by HSV. Administration may be by intravenous, intramuscular, subcutaneous, oral, suppository, mucosal, or topical routes. Also contemplated is DNA immunization wherein DNA encoding a gD variant molecule of the invention is provided to a mammal.

Brief Summary Paragraph Right (17):
It is also contemplated that the HSV gD variant molecules may act as immunogens in a mammalian recipient when administered by systemic or mucosal routes. Immunization of animals With wild type gD stimulates the production of virus neutralizing antibodies and protects them from lethal challenge with HSV-1 and HSV-2 [Long et al., Infect. Immunol., 37:761-764 (1984)]. The contemplated dual nature of the gD variant molecules is an advantage of the invention not shared by prior anti-HSV compounds discussed herein.

Detailed Description Paragraph Right (1):
Numerous variant HSV-1 molecules were constructed and analyzed for the ability to rescue infectivity of the gD-null virus F-gD/.beta. [Ligas and Johnson, J. Virol., 62:1486-1494 (1988)]. Variant molecules with mutations in one of four regions of gD (region I comprising amino acid residues 27 through 43, region II comprising amino acid residues 126 through 161, region III comprising amino acid residues 225 through 246, and region IV comprising amino acid residues 277 through 310) were unable to effect rescue. The four regions of gD-1 were thus determined to be necessary for entry of HSV into susceptible cells. Further analysis of representative variant molecules, each including mutations in one of the regions, identified a region IV gD variant molecule with particularly potent anti-vital activity.

Detailed Description Paragraph Right (12):
Full length variant gD molecules were expressed in COS-1 or L cells transfected with the genes described in Example 1 by a modified calcium phosphate-DNA coprecipitation method described in Cohen et al., supra. Full-length variants may also be expressed using baculovirus vectors in Sf9 cells [Landolfi et al., Vaccine, 11: 407-414 (1993)].

Detailed Description Paragraph Right (18):
Finally, to examine the functional characteristics of the variant gD-1 molecules, the ability of the variants to rescue the infectivity of the gD-null virus F-gD.beta. was assayed. The virus replicates in and forms plaques on VD60 cells which contain an integrated gD gene under the control of its own promoter. L cells transiently transfected with genes encoding a variant were then superinfected with F-gD.beta.. Pseudotype particles were harvested and litered on VD60 cells. The number of plaques measure the extent to which the variant gD molecule rescued the infectivity of the null virus. When infectivity was rescued with the wild type gD gene, the yields were typically 2.times.10.sup.6 PFU of progeny extracellular virus and 10.sup.6 PFU of intracellular virus. Virus yields from wild-type gD were considered to be 100%. The region I, II, III, and IV variant gD-1 molecules were able to complement the null virus in some cases only at very low levels and in other cases not at all.

Detailed Description Paragraph Right (22):
The ability of the variants to block HSV-1 infection of susceptible cells was measured by three assays diagrammed in FIG. 3, a plaque formation assay, a cell to cell spread assay, and an HSV-1/lacZ+ entry assay.

Detailed Description Paragraph Right (23):
The plaque formation assays were performed as described in Tal-Singer et al., supra. Briefly Vero or BHK cell monolayers in 48-well plates were treated with region I, II, III, or IV variant or BSA diluted in 5% DMEM for 1.5 hours at 4.degree. C. HSV-1 strain NS was added at 50 PFU per well for 1.5 hours and then treated with variant or BSA. After 24 hours at 37.degree. C., the medium was removed and the cells were fixed and air dried. Virus liters were determined by immunoperoxidase assay and the mount of variant needed to block HSV infection was titrated. Results of the assays for the truncated wild type gD-1 molecule and the gD-1 (.DELTA.290-299t) variant are presented in FIGS. 4A and 4B. If the amount of wild type gD-1(306t) needed to block infection by more than 60% is equal to 1, the region I variant gD-1(.gradient.34t) failed to block infection at all, the region I11 variant gD-1(.gradient.243t) blocked one-half as well as wild type, and the region II variant gD-1(.gradient.126t) blocked as well as wild type. In comparison to these results, a significant increase in ability to block infection was exhibited by the region IV variant gD-1(.DELTA.290-299t). It was able to block infection about 400 times better than wild type gD-1.

Detailed Description Paragraph Right (27):
In HSV/lacZ+ assays, confluent Veto cell monolayers in 96 well culture plates were preincubated with variant diluted in DMEM (BioWhittaker, Walkersville, Md.)+5% fetal bovine serum for 90 minutes at 4.degree. C. To each well, 1.5.times.10.sup.4 PFU/well of 7134 virus, an HSV-1 KOS strain with both copies of ICPO gene replaced by E. coli lacZ gene [Cal et al., J. Virol., 63:4579-4589 (1989)], corresponding to an MOI of about 0.4, was added for 90 minutes at 4.degree. C. After 4 hours incubation at 37.degree. C. the medium was removed and the cells were lysed with 0.5% NP40. The NP40 lysates were transferred to a new 96 well plate. .beta.-galactosidase activity was detected by adding CPRG (Boehringer Mannheim, Indianapolis, Ind.) substrate solution. Absorbance was measured at 570 nm using a Microplate Biokinetics Reader (Bio-Tek Instruments, Winooski, Vt.). Results of the assay are presented in FIG. 7.

Detailed Description Paragraph Right (29):
While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. For example, corresponding region IV variant gD-2 molecules and other region IV variant gD-1 molecules will be constructed and tested in the same assays for the ability to block HSV-1 and/or HSV-2 infection. Other variants specifically contemplated include, but are not limited to, variants comprising insertions at gD-1 residue 290, variants comprising insertions at gD-1 residue 300, variants comprising deletions of amino acids 277 through 290 and variants comprising deletions of residues

277 through 300. These variants may be made as either full-length or carboxy terminal truncated gD molecules. Accordingly only such limitations as appear in the claims should be placed on the invention.

Other Reference Publication (2):
Cai et al., "Herpes Simplex Virus Type 1 ICPO Plays a Critical Role in the De Novo Synthesis of Infectious Virus following Transfection of Viral DNA," J. Virol., 63(11):4579-4589 (1989).

Other Reference Publication (3):
Chiang et al. "Identification of Functional Regions of Herpes Simplex Virus Glycoprotein gD by Using Linker-Insertion Mutagenesis," J. Virol., 68(4):2529-2543 (1994).

Other Reference Publication (4):
Cohen et al. "Expression of Herpes Simplex Virus Type 1 Glycoprotein D Deletion Mutants in Mammalian Cells," J. Virol., 62(8):1932-1940 (1988).

Other Reference Publication (6):
Hill et al., "Trauma to the Skin Causes Recurrence of Herpes Simplex in the Mouse," J. gen. Virol., 39: 21-28 (1978).

Other Reference Publication (7):
Hill et al., "Adrenergically induced recurrent HSV-1 corneal epithelial lesions," Curr. Eye Res., 6(8): 1065-1071 (1987).

Other Reference Publication (8):
Johnson et al., "Soluble Forms of Herpes Simplex Virus Glyoprotein D Bind to a Limited Number of Cell Surgace Receptors and Inhibit Virus Entry into Cells," J. Virol., 64(6): 2569-2576 (Jun. 1990).

Other Reference Publication (10):
Landolfi et al., "Baculovirus-expressed herpes simplex virus type 2 glycoprotein D is immunogenic and protective against lethal HSV challenge," Vaccine, 11: 407-414 (1993).

Other Reference Publication (11):
Ligas and Johnson, "A Herpes Simplex Virus Mutant in Which Glycoprotein D Sequences Are Replaced by .beta.-Galactosidase Sequences Binds to but Is Unable To Penetrate into Cells," J. Virol., 62(5): 1486-1494 (1988).

Other Reference Publication (12):
Long et al., "Glycoprotein D Protects Mice Against Lethal Challenge with Herpes Simplex Virus Types 1 and 2," Infect. Immun. 37(2): 761-764 (1984).

Other Reference Publication (13):
Martin et al., "Soluble Glycoprotein D Blocks Herpes Simplex Virus Type 1 Infection of Rat Eyes," J. Virol., 66(9): 5183-5189 (Sep. 1992).

Other Reference Publication (15):
Muggeridge et al., "Identification of a Site on Herpes Simplex Virus Type 1 Glycoprotein D That Is Essential for Infectivity," J. Virol., 64(8): 3617-3626 (1990).

Other Reference Publication (16):
Rock and Fraser, "Detection of HSV-1 genome in central nervous system of latently infected mice," Nature, 302(7): 523-525 (1983).

Other Reference Publication (17):
Sisk et al., "High-Level Expression and Purification of Secreted Forms of Herpes Simplex Virus Type 1 Glycoprotein gD Synthesized by Baculovirus-Infected Insect Cells," J. Virol., 68(2): 766-775 (1994).

Other Reference Publication (18):
Stanberry et al., "Genital Herpes in Guinea Pigs: Pathogenesis of the Primary

Infection and Description of Recurrent Disease," J. Infect. Dis., 146(3): 397-404 (Sep. 1982).

Other Reference Publication (19):
Stanberry, L.R., "Pathogenesis of Herpes Simplex Virus Infection and Animal Models for its Study," Current Topics in Microbiol. and Immuno., 179: 15-30 (1992).

Other Reference Publication (21):
Stevens and Cook, "Latent Herpes Simplex Virus in Spinal Ganglia of Mice," Science, 173: 843-845 (1971).

Other Reference Publication (22):
Straus et al., "Suppression of Frequently Recurring Genital Herpes, A Placebo-Controlled Double-Blind Trial of Oral Acyclovir," N. Eng. J. Med., 310(24): 1545-1550 (1984).

Other Reference Publication (23):
Tal-Singer et al., "Interaction of Herpes Simplex Virus Glycoprotein gC with Mammalian Cell Surface Molecules" J. Virol., 69(7): 4471-4483 (1995).

Other Reference Publication (24):
Tenser, R.B., "Intracerebral Inoculation of Newborn and Adult Mice with Thymidine Kinase-Deficient Mutants of Herpes Simplex Virus Type 1," J. Infect. Dis., 147: 956 (May 1983).

Other Reference Publication (27):
Watson et al., "Herpes Simplex Virus Type-1 Glycoprotein D Gene: Nucleotide Sequence and Expression in Escherichia coli," Science, 218(22): 381-384 (Oct. 1982).

CLAIMS:

1. A variant herpes simplex virus glycoprotein D molecule comprising amino acids 1 to 300 of SEQ ID NO: 2.

10. A method for producing a variant herpes simplex virus glycoprotein D molecule comprising growing a host cell according to claim 9 in a suitable nutrient medium and isolating said protein from the cell or the medium of its growth.

(1990). The nucleotide sequence of the strain 333 gD-2 gene is set out in SEQ ID NO: 14 herein.

Brief Summary Paragraph Right (7):
The present invention provides variant HSV gD molecules capable of preventing infection of cells by HSV-1 and/or HSV-2. Also provided are novel purified and isolated polynucleotides (i.e., DNA and RNA both sense and antisense strands) encoding the variant gD molecules. HSV gD-1 and gD-2 region IV variants or fragments thereof are specifically contemplated by the invention.

Brief Summary Paragraph Right (9):
The presently preferred gD-1 variant molecule, designated gD-1(.DELTA.290-299t), is the product of recombinant expression in Sf9 cells of a fusion protein including the signal peptide of honeybee melittin [Tessier et al., Gene, 98: 177-183 (1991)] and Patton strain HSV-1 gD wherein (1) the Patton strain amino acid residues 290 through 299 of the mature gD-1 protein have been replaced with the amino acid residues arginine, lysine, isoleucine and phenylalanine, and (2) Patton strain amino acid residues 308 through 369 have been replaced with five histidine residues. When expressed in Sf9 cells, cleavage of the melittin signal peptide results in the presence of aspartate and proline residues at the amino terminus of the variant molecule. The amino acid sequence of gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 2 and the preferred DNA sequence encoding gD-1(.DELTA.290-299t) is set out in SEQ ID NO: 1.

Brief Summary Paragraph Right (15):
Administration of gD variant molecules or fragments thereof to mammalian subjects, especially humans, for the purpose of preventing HSV infection and/or ameliorating pathological sequelae of HSV infection is specifically contemplated. Various animal models for HSV infection are accepted in the art and include, but are not limited to, the rabbit and mouse eye models of herpes keratitis [Hill et al., Curr. Eye Res., 6: 1065-1071 (1987) and Rock and Fraser, Nature, 302: 523-525 (1983)], cutaneous herpes infection of hairless (nude) mice [Metcalf et al., Infect. Immunol., 26: 1164-1171 (1979)], vaginal lesions in the guinea pig and mouse [Stanberry et al., J. Infect. Dis., 146: 397-404 (1982)], foot pad model in mice [Stevens and Cook, Science, 173: 843-845 (1971)], zosterform skin model in mice [Hill et al., J. Gen. Virol., 39: 21-28 (1982)], and experimental herpes simplex encephalitis induced by intracerebral viral inoculation in mice [Tenser, J. Infect. Dis., 147: 956 (1983)]. For review, see Stevens, Microbiol. Rev., 53: 318-332 (1989) and Stanberry, Current Topics in Microbiol. and Immunol., 179: 15-30 (1992). The gD variant molecules are administered to the mammal in an amount sufficient to block infection of susceptible cells by HSV. Administration may be by intravenous, intramuscular, subcutaneous, oral, suppository, mucosal, or topical routes. Also contemplated is DNA immunization wherein DNA encoding a gD variant molecule of the invention is provided to a mammal.

Brief Summary Paragraph Right (17):
It is also contemplated that the HSV gD variant molecules may act as immunogens in a mammalian recipient when administered by systemic or mucosal routes. Immunization of animals with wild type gD stimulates the production of virus neutralizing antibodies and protects them from lethal challenge with HSV-1 and HSV-2 [Long et al., Infect. Immunol., 37: 761-764 (1984)]. The contemplated dual nature of the gD variant molecules is an advantage of the invention not shared by prior anti-HSV compounds discussed herein.

Drawing Description Paragraph Right (10):
FIGS. 9A and 9B are graphs respectively showing the results of plaque formation assays in which wild type gD-1(306t) and variant gD-1(.DELTA.290-299t) exhibited inhibitory effects on various HSV-1 strains;

Detailed Description Paragraph Right (1):
Numerous variant HSV-1 molecules were constructed and analyzed for the ability to rescue infectivity of the gD-null virus F-gD.beta. [Ligas and Johnson, J. Virol., 62: 1486-1494 (1988)]. Variant molecules with mutations in one of four regions of gD (region I comprising amino acid residues 27 through 43, region II comprising amino acid residues 126 through 161, region III comprising amino acid residues 225 through 246, and region IV comprising amino acid residues 277 through 310) were unable to

effect rescue. The four regions of gD-1 were thus determined to be necessary for entry of HSV into susceptible cells. Further analysis of representative variant molecules, each including mutations in one of the regions, identified a region IV gD variant molecule with particularly potent anti-viral activity.

Detailed Description Paragraph Right (2):
The following examples illustrate the invention wherein Example 1 describes the construction of vectors encoding variant gD-1 molecules of the invention, Example 2 describes recombinant expression of the variants in mammalian and insect cells, Example 3 presents results of analyses of the conformation of the variants and the ability of the variants to complement the null virus F-gD.beta., and Example 4 presents results of analyses of the ability of the variants to bind HSV susceptible cells and to block infection of HSV-susceptible cells by HSV-1 strains. Example 5 describes assays demonstrating the ability of variants of the invention to block infection by various other HSV-1 strains while Example 6 describes assays demonstrating that variants block infection of susceptible cells by HSV-2. Example 7 demonstrates the ability of variants of the invention to induce production of HSV neutralizing antibodies when the variants are utilized as immunogens. Example 8 describes methods for producing monoclonal antibodies specifically immunoreactive with variants of the invention.

Detailed Description Paragraph Right (12):
Full length variant gD molecules were expressed in COS-1 or L cells transfected with the genes described in Example 1 by a modified calcium phosphate-DNA coprecipitation method described in Cohen et al., supra. Full-length variants may also be expressed using baculovirus vectors in Sf9 cells [Landolfi et al., Vaccine, 11: 407-414 (1993)].

Detailed Description Paragraph Right (18):
Finally, to examine the functional characteristics of the variant gD-1 molecules, the ability of the variants to rescue the infectivity of the gD-null virus F-gD.beta. was assayed. The virus replicates in and forms plaques on VD60 cells which contain an integrated gD gene under the control of its own promoter. L cells transiently transfected with genes encoding a variant were then superinfected with F-gD.beta.. Pseudotype particles were harvested and titered on VD60 cells. The number of plaques measure the extent to which the variant gD molecule rescued the infectivity of the null virus. When infectivity was rescued with the wild type gD gene, the yields were typically 2.times.10.sup.6 PFU of progeny extracellular virus and 10.sup.6 PFU of intracellular virus. Virus yields from wild-type gD were considered to be 100%. The region I, II, III, and IV variant gD-1 molecules were able to complement the null virus in some cases only at very low levels and in other cases not at all.

Detailed Description Paragraph Right (22):
The ability of the variants to block HSV-1 infection of susceptible cells was measured by three assays diagrammed in FIG. 3, a plaque formation assay, a cell to cell spread assay, and an HSV-1/lacZ+entry assay.

Detailed Description Paragraph Right (23):
The plaque formation assays were performed as described in Tal-Singer et al., supra. Briefly Vero or BHK cell monolayers in 48-well plates were treated with region I, II, III, or IV variant or BSA diluted in 5% DMEM for 1.5 hours at 4.degree. C. HSV-1 strain NS was added at 50 PFU per well for 1.5 hours and then treated with variant or BSA. After 24 hours at 37.degree. C., the medium was removed and the cells were fixed and air dried. Virus titers were determined by immunoperoxidase assay and the amount of variant needed to block HSV infection was titrated. Results of the assays for the truncated wild type gD-1 molecule and the gD-1(.DELTA.290-299t) variant are presented in FIGS. 4A and 4B. If the amount of wild type gD-1(306t) needed to block infection by more than 60% is equal to 1, the region I variant gD-1(.gradient.34t) failed to block infection at all, the region III variant gD-1(.gradient.243t) blocked one-half as well as wild type, and the region II variant gD-1(.gradient.126t) blocked as well as wild type. In comparison to these results, a significant increase in ability to block infection was exhibited by the region IV variant gD-1(.DELTA.290-299t). It was able to block infection by HSV-1 about 400 times better than wild type gD-1.

Detailed Description Paragraph Right (24):

When variant gD-1(.DELTA.290-299t) was denatured by heating to 65.degree. C. for five minutes and then chilled to 4.degree. C. before the plaque formation assay was performed, the variant was incapable of blocking HSV-1 infection. Heat denaturation destroys secondary and tertiary structure of gD but leaves disulfide bonds intact. See FIG. 5.

Detailed Description Paragraph Right (26):
The cell to cell spread assay was performed in the same manner as the plaque formation assay, except that the gD proteins were not added until three hours after cells were infected with HSV-1. Results of the assays are presented in FIG. 6.

Detailed Description Paragraph Right (27):
In HSV/lacZ+assays, confluent Vero cell monolayers in 96 well culture plates were preincubated with variant diluted in DMEM (BioWhittaker, Walkersville, Md.)+5% fetal bovine serum for 90 minutes at 4.degree. C. To each well, 1.5.times.10.sup.4 PFU/well of 7134 virus, an HSV-1 KOS strain with both copies of ICPO gene replaced by E. coli lacZ gene [Cai et al., J. Virol., 63: 4579-4589 (1989)], corresponding to an MOI of about 0.4, was added for 90 minutes at 4.degree. C. After four hours incubation at 37.degree. C. the medium was removed and the cells were lysed with 0.5% NP40. The NP40 lysates were transferred to a new 96 well plate. .beta.-galactosidase activity was detected by adding CPRG (Boehringer Mannheim, Indianapolis, Ind.) substrate solution. Absorbance was measured at 570 nm using a Microplate Biokinetics Reader (Bio-Tek Instruments, Winooski, Vt.). Results of the assay are presented in FIG. 7.

Detailed Description Paragraph Right (29):
Baculovirus-produced region IV variant gD molecules of the invention were also examined in plaque formation assays as described in Example 4 for the ability to block infection of susceptible cells by different HSV-1 strains as well as two mutant HSV-1 strains, the infectivity of which is not blocked by wild type gD-1(306t). The various strains can be differentiated by comparison of their nucleotide and amino acid sequences.

Detailed Description Paragraph Right (30):
When the wild type gD-1(306t) and variant gD-1 molecule .DELTA.290-299t were tested in plaque formation assays in which HSV-1 strains HFEM, KOS, and 17 were utilized, the variant gD-1(.DELTA.290-299t) molecule exhibited an enhanced inhibitory effect on each strain in comparison to the wild type gD-1. Compare FIGS. 9A (306t) and 9B (.DELTA.290-299t). These results are consistent with the results presented for HSV-1 strain NS in the foregoing example. Moreover, the variant gD-1(.DELTA.290-299t) was able to inhibit infection by two mutant HSV-1 strains rid1 and rid2 (FIG. 10) while the wild type molecule could not. The rid1 and rid2 mutant strains were generated by passaging HSV-1 strain KOS on gD-expressing cells to select viruses that could overcome the ability of wild type gD-1 to inhibit infectivity of the viruses. See Dean et al., Virology, 199: 67-80 (1994). Neither wild type gD-1 (data not shown) nor the variant gD-1(.DELTA.290-299t) was able to inhibit infection by the mutant HSV-1 strain ANG (FIG. 9). The ANG virus strain is described in Dean et al., J. Virol., 69(8): 5171-5176 (1995) as a strain that is almost completely resistant to gD-mediated interference (i.e., the ANG virus can infect cells expressing gD).

Detailed Description Paragraph Right (33):
The ability of gD-1 molecules to inhibit other herpesviruses was also investigated. The gD-1 variant (.DELTA.290-299t) molecule was tested in plaque formation assays (Example 4) involving HSV-2 strain 333 and bovine herpes 1 (BHV-1), a related alphaherpesvirus. As shown in FIG. 11, the .DELTA.290-299t variant inhibited HSV-1 but not BHV-1, demonstrating that its inhibitory ability is specific for HSV infection.

Detailed Description Paragraph Right (35):
Polyclonal antibodies specific for wild type gD-1(306t) and the gD-1 variant (.DELTA.290-299t) molecule were elicited in rabbits. The polyclonal antbodies were then assayed for the ability to neutralize HSV-1 infectivity.

Detailed Description Paragraph Right (36):
Two rabbits (designated R122 and R123) were intially immunized with an inoculation of 100 .mu.g purified gD-1(306t), while two rabbits (designated R130 and R131) were initially immunized with an inoculation of 100 .mu.g purified gD-1(.DELTA.299-299t).

Both sets of initial inocula were mixed at a 1:1 ratio with Freund's complete adjuvant and given subcutaneously in inguinal and axillary regions and intramuscularly in hind limbs. No more than 0.2 ml per subcutaneous site and no more than 0.5 ml per intramuscular site were administered. After two weeks, each rabbit was boosted with 50 .mu.g of its respective antigen mixed at a 1:1 ratio with Freund's incomplete adjuvant. The first boosts (and all subsequent boosts) were given subcutaneously along the back and intramuscularly in the hind limbs. No more than 0.2 ml per subcutaneous site and no more than 0.5 ml per intramuscular site were administered. The rabbits were similarly boosted at weeks 3, 7, 10, and 14. Tests bleeds were taken at weeks 5, 8, 11, and 16. Neutralization assays were performed with polyclonal sera from the fourth bleed.

Detailed Description Paragraph Right (37):
The polyclonal antibodies were assayed for the ability to neutralize HSV-1 infectivity. The ability of the antibodies to inhibit infectivity indicates that the antigenic conformation of the variant gD molecule is similar to the wild type gD molecule. Wild type gD is known to induce production of potent virus-neutralizing antibodies when injected into animals.

Detailed Description Paragraph Right (38):
The rabbit sera were heat treated at 56.degree. C. for 30 minutes to inactivate complement. Vero cells were grown in 48 well plates until the monoplayer was nearly confluent. Serial two-fold dilutions of serum were prepared in DMEM medium containing 5% fetal bovine serum (FBS), then mixed with an equal volume of HSV-1 strain KOS in the same medium. The virus concentration was adjusted to give approximately 100 plaques per well of the 48 well plate in the absence of neutralizing antibody. Each virus-rabbit serum mixture was plated in duplicate onto the Vero cell monolayers and incubated for 1 hour at 37.degree. C. Each well of the 48 well plate was then overlaid with medium and incubated at 37.degree. C. until visible plaques developed. The medium was removed and the cells were fixed with a mixture of methanol and acetone and dried. Plaques were visualized by incubating the monolayers with a cocktail of antibodies to glycoproteins gD, gB and gC, then performing a "black plaque assay" using horseradish peroxidase conjugated protein A, followed by addition of the substrate 4-chloro-1-naphthol. Plaques were then counted. The results of the assays are presented in Table 2 below wherein the titer value is the dilution of antibody that reduced HSV-1 plaque number by 50%.

Detailed Description Paragraph Right (41):
To generate monoclonal antibodies, female Balb/c mice are immunized with 50 .mu.g of a variant gD-1 molecule. The immunogen is prepared in complete Freund's adjuvant, with subsequent boosts (25 .mu.g antigen in incomplete Freund's) at about 21 day intervals. Cell lines producing monoclonal antibodies are isolated as follows. Briefly a single cell suspension is formed by suspending immunized mouse spleen cells in serum free RPMI 1640, supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 .mu.g/ml streptomycin (RPMI) (Gibco, Canada). The cell suspension is filtered through sterile 70-mesh Nitex cell strainer (Becton Dickinson, Parsippany, N.J.), and washed twice by centrifuging at 200 g for 5 minutes and resuspending the pellet in 20 ml serum free RPMI. Thymocytes taken from three naive Balb/c mice are prepared in this manner.

Detailed Description Paragraph Right (46):
While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. For example, corresponding region IV variant gD-2 molecules and other region IV variant gD-1 molecules will be constructed and tested in the same assays for the ability to block HSV-1 and/or HSV-2 infection. Other variants specifically contemplated include, but are not limited to, variants comprising insertions at gD-1 residue 290, and variants comprising insertions at gD-1 residue 300. These variants may be made as either full-length or carboxy terminal truncated gD molecules. Accordingly only such limitations as appear in the claims should be placed on the invention.

Detailed Description Paragraph Table (1):
TABLE 1 IC.sub.50 (nM) Virus Strain gD-1(306t) gD-1(.DELTA.290-299t) Fold difference HSV-1 NS

1600 4.0 400 KOS 218 2.6 84 17 139 4.8 29 HFEM 180 16.4 11 rid1 NE 131 -- rid2 NE 150 -- HSV-2 333 164 1.5 109

Other Reference Publication (2):
Cai et al., "Herpes Simplex Virus Type 1 ICPO Plays a Critical Role in the De Novo Synthesis of Infectiuos Virus following Transfection of Viral DNA," J. Virol., 63(11): 4579-4589 (1989).

Other Reference Publication (3):
Chiang et al. "Identification of Functional Regions of Herpes Simplex Virus Glycoprotein gD by Using Linker-Insertion Mutagenesis," J. Virol., 68(4): 2529-2543 (1994).

Other Reference Publication (4):
Cohen et al. "Expression of Herpes Simplex virus Type I Glycoprotein D Deletion Mutants in Mammalian Cells," J. Virol., 62(8): 1932-1940 (1988).

Other Reference Publication (6):
Hill et al., "Trauma to the Skin Causes Recurrence of Herpes Simplex in the Mouse," J. gen. Virol., 39: 21-28 (1978).

Other Reference Publication (7):
Hill et al., "Adrenergically induced recurrent HSV-1 corneal epithelial lesions," Curr. Eye Res., 6(8): 1065-1071 (1987).

Other Reference Publication (8):
Johnson et al., "Soluble Forms of Herpes Simplex Virus Glycoprotein D Bind to a Limited Number of Cell Surface Receptors and Inhibit Virus Entry into Cells," J. Virol., 64(6): 2569-2576 (Jun. 1990).

Other Reference Publication (10):
Landolfi et al., "Baculovirus-expressed herpes simplex virus type 2 glycoprotein D is immunogenic and protective against lethal HSV challenge," Vaccine, 11: 407-414 (1993).

Other Reference Publication (11):
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CLAIMS:

1. A method for inhibiting the infection of susceptible cells by herpes simplex virus comprising contacting said cells with a composition comprising a variant herpes simplex virus glycoprotein D molecule comprising amino acids 1 to 300 of SEQ ID NO: 2 and an acceptable carrier.

2. A method for inhibiting the infection of susceptible cells by herpes simplex virus comprising contacting said cells with a composition comprising a variant herpes simplex virus glycoprotein D molecule comprising amino acids -2 to 300 of SEQ ID NO: 2 and an acceptable carrier.

3. A method for inhibiting the infection of susceptible cells by herpes simplex virus comprising contacting said cells with a composition comprising a variant herpes simplex virus glycoprotein D molecule comprising amino acids 1 to 306 of SEQ ID NO: 2 and an acceptable carrier.

4. A method for inhibiting the infection of susceptible cells by herpes simplex virus comprising contacting said cells with a composition comprising the gD-1(.DELTA.290-299t) molecule (amino acids -2 to 306 of SEQ ID NO: 2) and an acceptable carrier.

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A role for cell migration in the sexual transmission of HIV-1?

Zacharopoulos VR, Perotti ME, Phillips DM.

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The issue of how human immunodeficiency virus-1 (HIV-1) enters the body following sexual contact has been the subject of considerable controversy. Several possible routes for the initial infection have been suggested [1-6], including the possibility that the transmission is mediated by HIV-1-infected lymphocytes or macrophages in serum and female genital tract secretions, rather than by free virus. We recently reported that HIV-1-infected, activated primary monocytes can migrate between epithelial cells grown in confluent monolayer cultures in vitro [7]. We report here on experiments carried out in mice to test the hypothesis that mononuclear blood cells are capable of migrating through intact epithelia, and thus of carrying a virus into an animal. We placed double-stained, activated mononuclear blood cells into the vaginas of mice; four hours later, numerous double-stained cells were observed in the connective tissue beneath the vaginal epithelium and the iliac lymph nodes of the experimental mice. We speculate that such migration may be involved in the sexual transmission of HIV-1.

PMID: 9210380 [PubMed - indexed for MEDLINE]

Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination.

Bouvet JP, Belec L, Pires R, Pillot J.

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The induction of antibodies in vaginal secretions by systemic (intramuscular) immunization in humans was investigated by using the tetanus toxoid vaccine. Five women, 30 to 40 years old, were injected with a currently used dose of toxoid (40 IU), and serum, saliva, and vaginal secretion samples were collected on day 0 and on day 6 or day 10. All of these subjects had been previously vaccinated at least 5 years before; four were in good health, whereas one suffered from AIDS in clinical category B3. In most cases, analysis of specific antibodies in the vaginal wash showed a dramatic rise after boosting. These antibodies were primarily of the immunoglobulin G (IgG) isotype. The specific activity (ratio of antibody titer to IgG concentration) was shown to increase after the booster injection, irrespective of variations in the IgG level during the menstrual cycle. Comparison between serum and genital antibodies showed no difference in terms of both specific activity and level of avidity. These results demonstrate that parenteral injections can induce a systemic-derived antibody release in the vaginal fluid. Hence, systemic vaccinations can be efficient at the genital level and thus could reinforce or even replace a local vaccine.

PMID: 8063413 [PubMed - indexed for MEDLINE]

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- Nat Med. 1996 Oct;2(10):1054-5.

Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques.

Lehner T, Wang Y, Cranage M, Bergmeier LA, Mitchell E, Tao L, Hall G, Dennis M, Cook N, Brookes R, Klavinskis L, Jones I, Doyle C, Ward R.

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Prevention of sexually transmitted HIV infection was investigated in macaques by immunization with a recombinant SIV (simian immunodeficiency virus) envelope gp 120 and core p27 vaccine. In two independent series of experiments, we used the novel targeted iliac lymph node (TILN) route of immunization, aiming close to the iliac lymph nodes draining the genitorectal mucosa. Rectal challenge with the SIVmac 32H J5 molecular clone in two series induced total protection in four out of seven macaques immunized by TILN, compared with infection in 13 of 14 unimmunized macaques or immunized by other routes ($P = 0.025$). The remaining three macaques showed either a decrease in viral load (> 90%) or transient viremia, indicating that all seven TILN-immunized macaques showed total or partial protection ($P = 0.001$). Protection was associated with significant increase in the iliac lymph nodes of IgA antibody-secreting cells to p27 ($P < 0.02$), CD8-suppressor factor ($P < 0.01$), and the chemokines RANTES and MIP-1 beta ($P < 0.01$).

PMID: 8673922 [PubMed - indexed for MEDLINE]

1: AIDS 1998 Jan 1;12(1):1-10

Targeted lymph-node immunization with whole inactivated simian immunodeficiency virus (SIV) or envelope and core subunit antigen vaccines does not reliably protect rhesus macaques from vaginal challenge with SIVmac251.

Lu X, Kiyono H, Lu D, Kawabata S, Torten J, Srinivasan S, Dailey PJ, McGhee JR, Lehner T, Miller CJ.

California Regional Primate Research Center, School of Veterinary Medicine, University of California Davis, USA.

OBJECTIVE: Sexual transmission of HIV is the most common route of HIV transmission throughout the world. To prevent sexually transmitted HIV infection, a vaccine is urgently needed. A previous report demonstrated the targeted immunization of the iliac lymph nodes with simian immunodeficiency virus (SIV) subunits protects rhesus macaques from rectal challenge with SIV. We sought to determine whether this immunization strategy could protect rhesus macaques from vaginal challenge with SIV. DESIGN: Macaques were immunized with either whole-killed SIV or envelope and core subunit antigen vaccines. Using three independent groups, with three macaques in each group, macaques were immunized by the targeted iliac lymph-node (TILN) route, injecting the vaccine close to the iliac lymph nodes that drain the genital tract. RESULTS: The TILN immunization procedure induced high-titer SIV-specific immunoglobulin (Ig) G antibodies in serum in all animals and anti-SIV IgG and IgA antibodies in the cervicovaginal secretions of most animals. After a series of three or four TILN immunizations, the animals were intravaginally challenged with SIVmac251. All animals became virus isolation-positive, except one animal immunized with SIV p27 and gp120. This animal was virus isolation-negative but SIV DNA proviral sequences were detected in peripheral blood mononuclear cells. CONCLUSIONS: In this series of studies, reliable protection from vaginal transmission of SIV was not achieved by the TILN immunization procedure.

PMID: 9456249 [PubMed - indexed for MEDLINE]

A rational basis for mucosal vaccination against HIV infection.

Lehner T, Bergmeier L, Wang Y, Tao L, Mitchell E.

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The lack of success in the development of an effective conventional vaccine against HIV has focused attention on mucosal immunity. This is a rational move, since HIV is transmitted mostly by the mucosal route. The mucosal strategy is based on the concept that: a) HIV/SIV has to cross the mucosal-regional lymph node-blood barriers, each of which can prevent viral transmission or decrease the viral load. b) Immunization has to target directly the mucosal tissues or indirectly the regional lymph nodes, in order to prevent or control viral replication. This strategy is consistent with antigen localization and effective entry into the lymph nodes, driving the immune response. c) A dual immune mechanism may be necessary for effective mucosal protection, mediated by specific CD4 and CD8 T-cell and antibody responses to the immunizing antigens, and innate antiviral factors and beta-chemokines which down-modulate CCR5 co-receptors. Targeted iliac lymph node immunization with SIVgp120 and p27 in alum prevents SIV infection or significantly decreases the viral load when challenged by the rectal route. Indeed, in addition to specific immunity, including significant sIgA antibody-forming cells in the iliac lymph nodes, CD8-suppressor factor and the three beta-chemokines (RANTES, macrophage inflammatory protein (MIP)-1 alpha and MIP-1 beta) are significantly associated with protection against rectal mucosal SIV infection.

Publication Types:
- Review
- Review, Tutorial

PMID: 10566151 [PubMed - indexed for MEDLINE]